

Research advances in genetics and breeding of *Populus davidiana* Dode in China

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Abstract In this paper a general introduction is given to research advances in genetics improvement and breeding of Chinese aspen (*Populus davidiana* Dode) in China. This introduction includes natural distribution and collection, conservation, gene diversity, provenance trial, crossing breeding, vegetative propagation and disease resistant etc. Based on the current situation of forest tree breeding in China, some strategic suggestions concerning the future development of Chinese aspen genetics improvement in China are presented, taking into consideration the existing domestic demands of forestry production and international trends in forest tree breeding.

Key words: Chinese aspen, Natural distribution, Genetic resources conservation, Cross breeding, Tissue culture, Vegetative propagation, Disease resistant

Introduction

Breeding of Chinese aspen (*Populus davidiana* Dode) in China started in 1950's when the first crossings were made by Professor Yi Peizhong in 1946 at the agriculture college of China center (Yi 1955) and Xu Weiyong in 1954 at the Chinese forestry research institute (Xu and Huang *et al.* 1956). Crossing was made between *Populus hopeiensis* and *P. davidiana*, *P. davidiana* and *P. simonii*. During the period 1960-1970, great attention was paid to the hybridization between *Populus* genus or races (Han 1995). The most effective, systematic research work began to develop since *P. davidiana* was taken as a national key research program in 1983 (Liu and Zhao 1991). This program covered distribution investigation, selection and collection, genetic resources conservation, vegetative propagation, crossing breeding etc. In the early 1990's, during the eighth "Five-year-plan", the program aimed at developing better planting material intended for the development of short-rotation industrial forests (Li and Xing *et al.* 1997). Not only tree growth characters, but also wood quality traits were considered. The program covered provenance trial, selection from the family. It seems that in the 1990s there was a rush for carrying out research with introducing of *P. tremula* and its triploid from abroad (Xu

and Liu *et al.* 1989; Zhang and Liu *et al.* 1991; Li and Fang 1992; Yang and Wang *et al.* 1995), *P. tremuloides*, vegetative propagation and variation on wood quality.

Chinese aspen is the most widely distributed tree in the China and exhibits considerable variation in form, growth rate, and wood properties. Wide geographic distribution has resulted in the description of a number of varieties. Chinese aspen has good form, rapid growth and improved fiber properties. Research on natural variation and heredity suggests considerable genetic gain will result from tree improvement programs emphasizing height and diameter growth and such wood properties. Properly oriented genetics and intensive management, and utilization can be expected to make aspen as even more valuable resource in the future.

Natural distribution and collection of gene resources

Chinese aspen is a pioneering species which is widely distributed, and well adapted, and has good quality of timber. It distributes from Ximalaya Mountains, to Daxing'an Mountains and Xiaoxing'an Mountains and from Artai Mountains to Wushulijiang river, covering 23 provinces of China (Zheng 1985). Usually it is not limited by soil condition and can adapt variable ecological conditions. It can also grow

in either drought site or fertile soil, either plain or alpine. It can regenerate from seeds or root-sucker. In the Daxing'an Mountains, *P. davidiana* forest takes an area of 180 000 hm², most of which are less than 25-35 year old originated from secondary stand mixed with birch, oak and larch etc. After the great forest fire in 1987, the area regenerated with the species covered over half of the burned land. In the Xiaoxing'an Mountains, *P. davidiana* has an area of 16 000 hm². Most of them are at the age of 25~35 a mixed with maple, lime and hard broad leaf species. *P. davidiana* growing in the Wanda mountains are superior (Liu and Zhao 1991; Liu and Li *et al*, 1995).

During 1980's, a large scale plus tree selection was carried out for Chinese aspen in all provinces (regions), a total of 793 plus trees were selected from 29 sites all over China, studied and utilized for the gene resources according to the climatic zones. Criteria and methods of selection have been developed. The main traits used in the selection of superior trees are both quantitative and qualitative. The candidates were evaluated for growth, morphology, quality and resistance and the method of simple index used in comparison. The candidate with a total score above mean value was regarded as plus tree (Zhao and Chen *et al* 1993).

Conservation of gene resources

All the plus trees were conserved in situ, and three natural gene banks were established in 1990 (Liu and Zhao 1991; Liu and Li *et al* 1993). The three natural gene banks are located on Hekou, Dayake, and Xi-angyang forest farm respectively. The first covered an area of 6.67 hm² at age 41a and 15 plus trees were selected; the second covered an area of 6.67 hm² at age 63 and 10 plus trees were selected; the third covered an area of 8 hm² at age 30.

Three artificial gene banks were established by grafting. The first two are located on Hekou forest farm with 134 clones (2 hm²), the second with 80 clones (1 hm²) respectively, and the third is located on Nanlin forest farm with 25 clones (1 hm²). The collective area was established on Jiangshanjiao forest farm and 104 clones were used and occupied 1 hm². A clone archive (1 hm²) with 206 clones was established in Dongfanghong forest bureau (Liu and Zhao 1991; Liu and Li *et al* 1993).

Variation types and gene diversity

Several varieties of *P. davidiana* have been described and found in the different areas. *P. davidiana* Dode var. *tomentella* Nakai was found at the elevation of 2 300~3 000 m in the mountains of Sichuan and Yunan Provinces of China, *P. davidiana* Dode var.

Pendula in Heilongjiang Province, *P. davidiana* dode f. *Laticuneata* in the south of Liaoning Province, and *P. davidiana* Dode f. *Ovata* C. Wang *et al* S. L. Tung in Ganshu, Shanxi and Hebei (Zheng 1985).

Taxonomists have divided the genus *Populus* into five sections. *Populus davidiana* Dode, along with the closely related species of European aspen (*P. Tremula* L.), Quaking aspen (*P. tremuloid* Michx.) and big-tooth aspen (*P. grandiden* Michx.) etc., have been placed in the subsection trepidae of the section leuce (FAO 1958).

There are also several types of *P. Davidiana* in the northeast area of China, such as Green-bark aspen, gray-bark and fire-aspen (Li and Wang 1993). Among them green-bark aspen grows fastest.

Based on observation in 50 fixed stands for 4 years, 14 morphological types of *P. davidiana* were temporarily classified on the features of typical leaves collected in the middle of crown (Chai 1993). They were cordifolia, microphylla, crenulata, longlamenta, latiovata, ramosissima, viridis, versicolor, saliciformis, oblata, densiserrata, grandiserrata, pauciserrata, and orbiculata.

The genetic variation among *P. davidiana* was detected by isoenzyme (Li and Wang *et al* 1993). The genetic diversity of clones which are from 12 provenances of *P. davidiana* was studied using seed protein in 1993 (Pang and Wang *et al* 1993). 13 provenance of *P. davidiana* were analyzed by POD isoenzyme (Wei and Hu *et al* 1997). Result indicated there are large diversity in Fanzheng, Dailing and Dongfanghong provenance.

Provenance trial

Provenance trials of *P. davidiana* started in the early 1990's in China. Based on the investigation all over China, 427 gene materials were collected from 34 forestry farms of 5 provinces (region) in China. And provenance trials at Yingchun, Fangzheng and Dailing forest farms of Heilongjiang province were established during the period 1991-1995 (Li and Xing *et al* 1997). The plantation was evaluated for growth, morphology, wood quality and resistance. 7 superiors in provenance and 14 superiors in family were selected by ANOVA, LSR and Synthesis Index in Heilongjiang Province.

Test plantation for provenance and family was established in Jiangbei Forest Farm and Shuiqiliu Forestry Farm of Jilin Province in 1993, spaced by 1 m × 2 m, 20 trees in a plot, replicated 4 times. 2 superiors (Fangzheng and Nantai provenance) and 4 superiors in family were selected in Jilin Province (Li and Zhang *et al* 1998). They were increased in tree height by 56.2% and 53.6%, in diameter growth by 95.2% and 79.5%, respectively. 4 superiors in family were

selected from 29 families. Fangzheng 4, which is 4.14 m in tree height and 2.44 cm in DBH at 4 years old, is the best of them and increases by 92.7% in height and 141.9% in DBH as compared with CK (Jilin provenance).

In Pingquan forest farm of Hebei Province, 3 superiors in provenance were selected in Hebei Province (Dai *et al* 1995), increased in tree height by 189.9%~142.2%. 10 superior families were selected and increased by 177% in height and 143% in diameter as compared as CK.

Cross breeding

Chinese aspen, because of its economic importance and because of the ease with which it can be crossed with other species of *Populus*, has been widely used as a parent in experimental crossing research. Some of the earliest descriptions of the using Chinese aspen as a parent tree include reports by Professor Yi Peizhong (1955) and Professor Xu Weiyong (1956). Crossing was made between *P. hopeiensis* and *P. davidiana*, *P. davidiana* and *P. simonii*. Liu Peilin *et al.* (1991) described several *P. alba* by *P. davidiana*. Early successful crossing and rapid growth was *P. alba* by *P. davidiana* and *P. davidiana* by (*P. alba* by *P. davidiana*) hybrids (Liu and Zhao 1991; Zhang and

Copes *et al* 1995).

A review of the literature indicates Chinese aspen has been successfully crossed with a large number of species of *Populus*, including species within the sections of Leuce, Aigeiros, and Tacamahaca. Table 1 summarizes the reported successful crosses along with remarks on the ease of crossing. Chinese aspen crosses readily within the section leuce and with difficulty with species in the other sections of genus *Populus*. Based upon growth rate and form, the most promising type of hybrids are those originating from crosses between *P. davidiana* and *P. tremuloides*, *P. alba*.

Using 44 female collected from 22 sites and 59 male trees from 26 sites, 257 mating combinations produced seeds (Liu and Zhao 1991). The seeds were sowed in Jiangshanjiao Forest Farm. Index of cold damage (ICD) of the seedlings increased as the seedlings increased as their parents were collected at lower latitude. ICD was 0 at latitude 45° N and it increased by 4% when the parents originated at latitude 1° less. When female parents derived from high latitudes were crossed with male parents from low latitudes. ICD of the hybrids decreased in comparison with the hybrids whose male males from high latitudes, ICD of the hybrids were slight higher.

Table 1. Hybrids having Chinese aspen parentage

Type of cross	Ease of crossing	Remarks
<i>P. hopeiensis</i> × <i>P. davidiana</i>	Easy	Yi Peizhong in 1946 (Yi 1955)
<i>P. davidiana</i> × <i>P. simonii</i>	Difficult	Xu Weiyong and Huang Dongsun in 1954 (Xu and Huang <i>et al.</i> 1956) and Li Kailong in 1995
<i>P. davidiana</i> × <i>P. alba</i>	Easy	Some crosses promising. See Liu Peilin (1991, 1995)
<i>P. alba</i> × <i>P. davidiana</i>	Easy	Very promising; reported by Liu Peilin (1991,1995) and Zhang Tingzhen (Zhang and Copes,1995)
<i>P. davidiana</i> × <i>P. tremula</i>	Easy	Some crosses promising; reported by Liu Peilin (1991,1995)
<i>P. tremula</i> × <i>P. davidiana</i>	Easy	Some crosses promising; reported by Liu Peilin (1991,1995)
<i>P. tremuloides</i> × <i>P. davidiana</i>	Easy	Moderate growth rate; reported by Heimbürger(1958) and Einspahr (1964)
<i>P. davidiana</i> × <i>P. tremuloides</i>	Easy	Very promising; done by Li kailong in 1995
<i>P. tomentosa</i> × <i>P. davidiana</i>	Easy	Promising; done by Li kailong in 1998
<i>P. davidiana</i> × <i>P. tomentosa</i>	Easy	Proming, done by Zhang Tingzhen (Zhang and Copes 1995)

General combining ability was analyzed based on the unbalanced mating design. *P. tremula*, *P. alba*, *P. davidiana* collected from Chuxiong, Dailing, Jiangshanjiao are higher GCA for racial hybrids. Female from *P. alba* are the best among the species and so are those from Jiangshanjiao and Chuxiong among races.

Using 11 female of *P. davidiana* from 7 sites and 8 male of *P. tremuloides* (T-6-61, T-28-56, T-20-60, T-201-68, Ta-10, T-44-60, T-32-57, T-12-67), 28 mating combinations produced seeds in 1995. The seeds were sowed in Harbin and their seedlings were

propagated as materials. The plantlets of nutrition container produced by tissue culture were planted in 1997. Results in investigated in 1998 shows in Table 2.

Vegetative propagation

The most common method by which Chinese aspen reproduces itself in the field is by suckering from lateral tree roots (Wang 1993). Vegetative shoots arise from adventitious buds on roots that are frequently less than 2 to 3 cm in diameter and growing

within a few centimeters of the soil surface. If left undisturbed, one clone can theoretically perpetuate itself indefinitely by root suckers.

Table 2. Growth of hybrids between *Populus davidiana* and *P. tremuloides*

Hybrids	Survive rate %	Heighth /cm	Diameter at root collar /cm
X3×T-1-96	71.4	116.15	0.89
X2×T-10-60	100.0	139.2	1.08
X2×T-10-60	60.0	154	1.22
X2×T-10-60	80.0	174.83	1.23
X2×T-10-60	100.0	223.25	1.56
G3×T-1-96	95.0	203.94	1.7
G3×T-1-96	90.5	223.05	1.73

Note: all female parent trees are *Populus davidiana*; all male parent trees are *Populus tremuloides* from Minnesota of America.

The root-ability of Chinese aspen dormant stem cuttings is very poor compared to the relative ease of rooting other species of *Populus* (Piao and Liu *et al* 1993). After storage for periods of 3 and 6 months at low temperature in the dark, treatment included quick basal dips in 0-500 ppm NAA or 0-700 ppm IBA or 0-1000 ppm ABT (rooting powder).

C. Muhle Larsen (1943) developed the technique of propagating root sprouts by planting isolated root sections of European aspen in moist peat in an unheated greenhouse. Root sprouts were excised when 3 to 5 cm tall and were rooted as green-wood or succulent cuttings. Yan Juexing (1993a), Zhang Guolian (1991), Li Fuqiang *et al* (1993) used this method successfully in Chinese aspen and it is now common technique.

Yan Juexing (1993b) grafted Chinese aspen scions into the trees of *P. Nigra* var. *Thevestina*, *P. Canadensis* and *P. Pseudo-simonii* etc. Europe aspen buds were successfully grafted onto Chinese aspen (Zhang and Liu 1991). The buds of *P. davidiana* were collected for tissue culture during dormant or germination period. 60% differentiation rate was obtained and each bud was able to produce 12-15 plantlets. Suitable medium for tissue culture is WM1+BA0.3+NAA0.01+ Glu 20 mg/l + Arg 10mg/l + sugar 2-3%, In the medium the differentiation percent from triploid was 80%, in 3 months plantlets reached 10 cm high. 14 plantlets were obtained from triploid *P. davidiana*, 8-9 plantlets from hybrid, and 6 plantlets from original one (Xu and Liu 1993).

The buds collected from one-year-old seedling with 80% differentiation rate produced 56 plantlets/100 days; from current shoots of seedling – 50-60% differential rate and 32-56 plantlets/100days; from current shoots of adult tree – 40-50% and 21-30 plantlets, and from old shoots of adult- 20-30% and 8-16 plantlets respectively.

AM or MS +KT 1.0mg/l + BA 0.3-0.5mg/l +NAA 0.05-0.1 mg/l + sugar 2-3%medium was used for successive generation culture, differentiated plantlets continued to be cultivated until 20 generation. After 2-3 generation successive culture (60-90 d), the plantlets were transferred to the medium of promoting shoot (1/2 MS+KT0.5+ BA0.3+NAA0.5), and then to the medium of inducing rooting and finally cultivated in the field.

A root diameter of 2 cm or so was suitable for root propagation. On average, each root produced 11.9 plantlets, but if the diameter was over 4 cm, only 0.7 plantlet would be produced. Soaked in NAA 100ppm + sugar 20% for several seconds, of 30% soft cuttings from adult plants could survive. Cuttings from current germinated shoots were soaked in 100 ppm ABT (rooting powder) for 20 minutes, then the survival rate was 60%.

Vegetative propagation is crucial for genetic improvement. At present only a small quantity of plantlets can be produced with tissue culture, grafting, roots and cuttings. Much is expected to done so as to be capable of propagating it in a large scale.

Disease resistance

Aspen root rot researches was carried out on identification of its pathogeny and biological characteristics, antagonism fungi confrontation experiments, chemistry medicines' selection and practice experiments in laboratory and field, the space distribution pattern of the disease etc (Xue Yu *et al* 1998, unpublished).

Aspen root rot disease is caused by *Fusarium acuminatum*, the pathological suitable growing temperature is 20-25°C in PDA culture medium, the best pH is 6-7, the conidial germination's suitable temperature is 10-15°C, the best growing carbonic resources are lactose and amylum, the best nitrogenous resource is KNO₃.

Chaetomium sp. And *Trichoderma* sp. Have strong antagonism effect against *F. Acuminatum* in the laboratory and the field experiments, the both of fungi are good one of biological medicines.

"Senbao No. 1", FeSO₄, 901 and "Cushengii" can control better aspen root rot and diseased aspen's density becomes lower. It is assembly pattern, when the diseased aspen's density increasing, the space distribution pattern gradually changes into random pattern; when average of disease aspen in sample field is bigger than its variance, then it's even distribution pattern.

Aspen root rot disease's space distribution pattern is relatively static. When the diseased aspen's density increasing, the space distribution pattern gradually changes into random pattern; when average of disease aspen in sample field is bigger than its vari-

ance, then it's even distribution pattern.

Outlook

Although some progress and achievements have been made on the genetic improvement and tree breeding of Chinese aspen in China, some shortages or questions remain in this field, such as a lack of breeding strategy or breeding plan for Chinese aspen. Too much effort has been spent solving today's problems and not enough consideration has been given to how future wood and paper product developments, and predicted land use trends affect forest tree improvement goals. After working for a number of years in a research area that lies at the interface between forest genetics, pulp and paper technology, the following aspects should be emphasized in the future research on the genetic improvement and breeding of Chinese aspen in China:

1) Maximum per hectare per year cellulose production through improved height and diameter growth, improved form (natural pruning, straghtness, and narrow crown), and vigorous suckering;

2) High juvenile wood specific gravity for greater pulp yield per cord and improved tearing strength;

3) Improved insect and disease resistance with special emphasis on hypoxylon canker and wood borers of the genus *Saperda* and *Agrilus*;

4) Available several methods of tree improvement should be used, the most promising of which are selection, hybridization, and polyploidy. Greatest gains can be expected from the use of combination of methods.

5) An elite gene pool of rent selection and progeny testing system should be established. Then, using the improved diploid material, it would be possible to produce new genetic combinations using polyploidy, interspecific hybridization, and combinations of hybridization and polyploidy.

6) In the improvement practice the traditional breeding should still be the backbone meanwhile active to develop the molecular genetic and the application of biotechnology in tree breeding.

7) More emphasis should be placed on stress resistance breeding for the selection and breeding of insect resistant planting material, disease resistance or drought resistance. The same emphasis ought to be placed on the sexual hybridization and the vegetative propagation of breeding results based on multiple generation breeding. The progress of clone breeding ought to be accelerated by being based on the specific propagation characteristics.

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